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EVOKED POTENTIALS IN IMMOBILIZED CATS  
TO A COMBINATION OF CLICKS WITH PAIN-  
FUL ELECTROCUTANEOUS STIMULI

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EVOKED POTENTIALS IN IMMOBILIZED CATS TO  
A COMBINATION OF CLICKS WITH PAINFUL  
ELECTROCUTANEOUS STIMULI

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Averaged, evoked potentials in the auditory, somatosensory and motor cortical zones, as well as in the mesencephalic reticular formation were recorded in acute experiments on nonanaesthetized immobilized cats. Combination of clicks with a painful stimulus enhanced in some animals the response to the click, in others reduced it, and in others the response did not change.

Omission of the painful stimulus after a number of pairings resulted in the appearance in its temporal interval of a so-called delayed evoked potential, often resembling in form the late phases of the response to the painful stimulus. It was most pronounced in the auditory cortex and the reticular formation. Its appearance in the motor area was linked to the end of the curare action.

The characteristics of this response are discussed in comparison with conditioned changes of the sensory potential amplitudes.

The study of electrophysiological phenomena opens up new approaches to an interpretation of the nature of reactions of

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\* Numbers in margin indicate pagination in original foreign text.

individual structures and the interaction of different cerebral formations in the process of working out a temporal link. One of the informative approaches is recording evoked potentials (EP) in the formation and realization of a conditioned reflex [2,4,12,13,19].

The majority of the known published EP studies have been conducted under conditions of a chronic experiment on nonanaesthetized animals. This method with unquestionable numerous advantages impairs an analysis of EP changes after local application of pharmacological agents, as well as with the action of amnesia factors such as narcosis or electroconvulsions. It was expedient to conduct EP studies in acute experiments with the maximum preservation of the procedure of conditioning.

This work studied the EP transformations in combination of indifferent (clicks) and reinforcing (painful electrocutaneous irritation) stimuli in experiments on nonanaesthetized and curarized cats. The selected model of the experiment with certain lag of the unconditioned stimulus from the conditioned made it possible besides the EP changes to the conditioned stimulus associated with the combination of irritations to record the so-called delayed evoked potential that emerges with time at the site of reinforcement, when the latter is omitted. This potential is observed when a click or electrical stimulation of the splanchnic nerve is used as the conditioned stimulus [8,9]. It is also found in the chronic experiment [10] which confirms the adequacy of the selected model.

#### Technique

The experiments were conducted on 65 nonanaesthetized cats immobilized by periodically administered gallamic triethiodide (Remiolan, "Spofa"). The operational technique was to insert cannulae into the vein and trachea, fix the head of the animal

in a stereoscopic instrument and expose the cortical surface--carried out under ether. The experiment started in 2 h after removal of the narcosis. The wound surfaces were periodically anaesthetized with a 0.25% solution of novocaine.

Monopolar recording of the EP was used at the focus of maximum activity (in several experiments next to it) of the motor (GSA), somatosensory (GSP) and auditory (ESM) parts of the cerebral cortex, as well as in the region of the mesencephalic reticular formation with coordinates according to the atlas of Snider and Niemer [20] A=1, L=2 and H=2. Silver electrodes were used with diameter of the end 0.5 mm. The subcortical points of contact were also were also monopolar with the help of Nichrome wires 0.3 mm insulated over the entire length except the end. The indifferent electrode was fastened in the frontal bone of the skull. The animal was in a sound-proof chamber.

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A click (2 ms, 45 db above human audibility) was used as the conditioned stimulus, and the unconditioned--a series of five current impulses from an ESU-1 stimulator (1 ms, 3-4 ma, 100 imp/s) supplied through subcutaneous needles to the anterior extremity of the contralateral zone of recording. The unconditioned stimulus (US) was made within 300, 500 or 1000 ms after feeding of the conditioned stimulus (CS).

The experimental program included: 1) isolated presentation of 60-100 CS--habituation; 2) combined presentation of 200 CS and US--training; 3) two combinations of CS and US alternating with two isolated CS--control (up to the set of 20 isolated CS); 4) in 59 experiments after one-two controls suppression followed (up to 500 isolated CS), in 7 experiments combinations of CS and US were presented with greater or lesser setting aside for "re-learning."

In the majority of experiments the stimuli were distributed randomly in time according to Poisson's law with mean frequency of presentation 1 in 7 s. UBP2-03 amplifiers were used with band 0.3-500 Hz, the EP was averaged for 20 by the ART-1000 analyzer. In the control only the EP was averaged for the isolated CS. Control by the ART-1000 analyzer and the stimulators was implemented by a semiautomatic programmer developed in the laboratory especially for the given experiments.

### Study Results

The characteristic responses of different zones of the cerebral cortex and the reticular formation (RF) to the conditioned and unconditioned stimuli with indication of the latent periods of peaks of individual phases are presented in figure 1. Table 1 presents the limit values of amplitudes of the EP phases numbered on fig. 1 (in the numerator of the fraction) and the percentage of experiments (in the denominator of the fraction) in which these phases were recorded. The data of each experiment were not statistically processed due to the loss of values of individual EP in the averaging on the ART-1000 and the small number of controls.

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Since our experiments used an unconditioned stimulus of great strength and duration, the response to it is difficult to compare to that described in the literature. The great removal of the US from the CS and the long response to the US required an increase in the period of synchronous accumulation on the ART-1000 to 1 s. This reduced the accuracy of the signal presentation and deprived us of the possibility of analyzing the characteristics of the group of early components of the response to the US, therefore the numerical material is given only for the late phases of responses.

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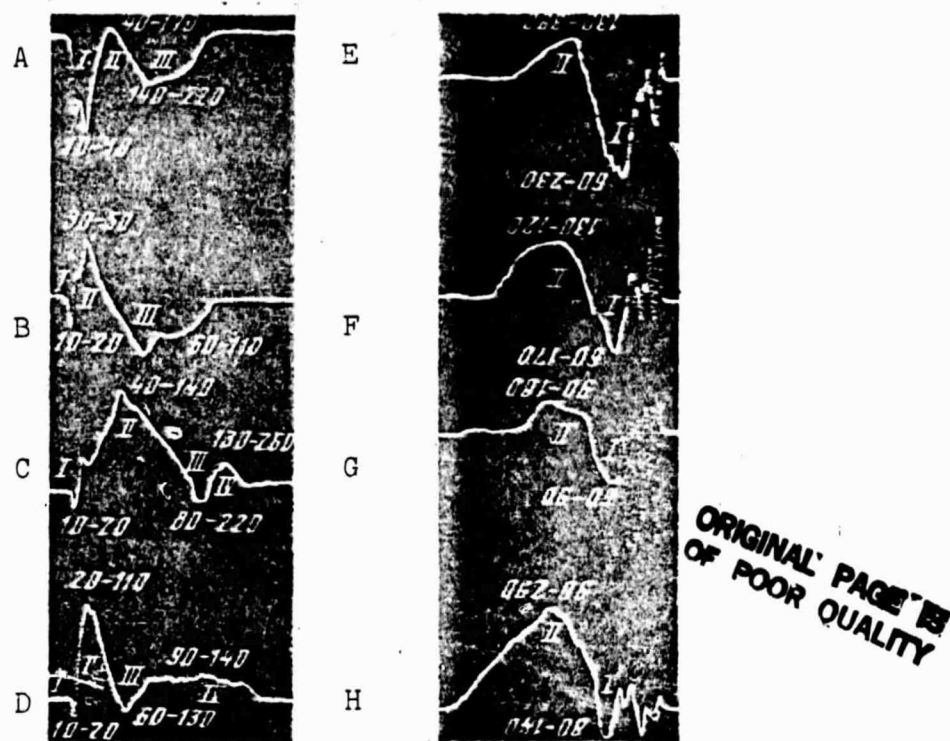


Figure 1. Characteristic Evoked Potentials to Click and Electrocutaneous Stimulation before Combination of Stimuli. On the left--EP to click, on the right--EP to electrocutaneous stimulation. A, E--auditory cortex; B, F--somatosensory cortex; C, G--motor cortex; D, H--reticular formation. Roman numerals--number of phases, arabic --limit values of latencies in the phase peak.

Averaged evoked responses to conditioned and unconditioned stimuli. The latencies of the phase peaks and the overall length of the EP to clicks in the procedure of "training" did not significantly change in any of the recording regions.

In the auditory zone of the cortex the EP to the CS (fig. 1, A) in different experiments lasted 120-330 ms. The positive phase I, as a rule, was represented by two components. After combinations of CS and US the late component of this phase was altered. Changes in phases II and III statistically reliably ( $D < 0.01$ ) were unidirectional (with an increase or decrease in phase II correspondingly

TABLE 1  
AMPLITUDE OF EP TO ISOLATED PRESENTATIONS OF CLICK AND ELECTRO-  
CUTANEOUS STIMULATION IN DIFFERENT REGIONS OF THE BRAIN (  $\mu v$  )

Region of point of contact	Stimulus	EV Phases			
		I	II	III	IV
Auditory zone	Click	$\frac{110-500}{100}$	$\frac{50-160}{86}$	$\frac{10-260}{86}$	
	Electrocutaneous stimulus	$\frac{10-200}{38}$	$\frac{20-300}{92}$		
Somatosensory zone	Click	$\frac{20-110}{74}$	$\frac{20-100}{44}$	$\frac{10-140}{25}$	
	Electrocutaneous stimulus	$\frac{20-300}{89}$	$\frac{50-200}{66}$		
Motor zone	Click	$\frac{10-180}{90}$	$\frac{10-140}{80}$	$\frac{10-160}{52}$	$\frac{10-80}{24}$
	Electrocutaneous stimulus	$\frac{30-400}{55}$	$\frac{20-160}{55}$		
Reticular formation	Click	$\frac{10-120}{69}$	$\frac{10-250}{100}$	$\frac{10-130}{52}$	$\frac{10-80}{19}$
	Electrocutaneous stimulus	$\frac{10-130}{64}$	$\frac{40-100}{36}$		

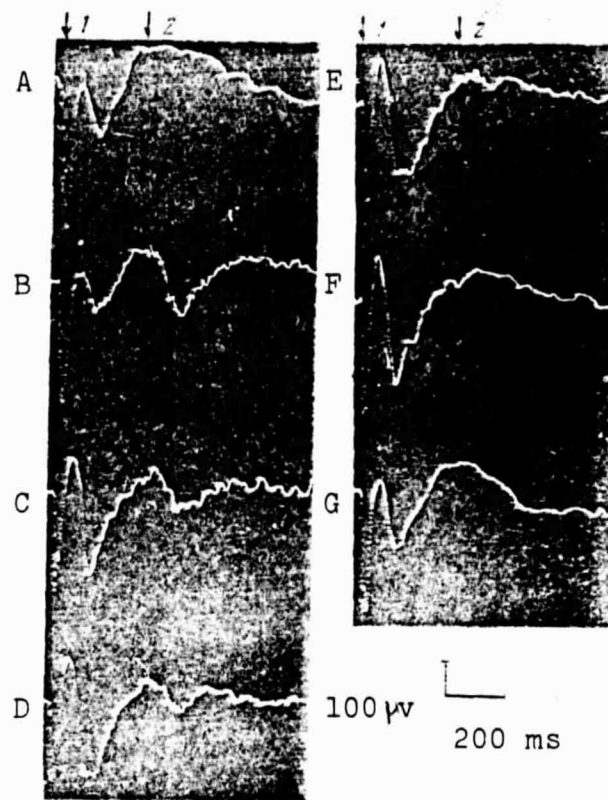
Note: Explanations in text

TABLE 2  
CHANGES IN EP AMPLITUDE OF AUDITORY ZONE OF CORTEX AFTER COM-  
BINATIONS OF CLICK AND ELECTROCUTANEOUS STIMULUS

Stimulus	Nature of changes in amplitude	EP Phases		
		I	II	III
Click	Reduction	$\frac{30}{23}$	$\frac{80}{28}$	$\frac{72}{31}$
	Increase	$\frac{30}{23}$	$\frac{185}{24}$	$\frac{182}{31}$
Electrocutaneous stimulus	Reduction	$\frac{100}{26}$	$\frac{30}{16}$	
	Increase	$\frac{216}{31}$	$\frac{41}{34}$	

Note: In numerator--mean percentage of change in amplitude, in denominator--percentage of experiments in which change was recorded.





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Figure 2. DEP in Auditory Region of Cortex and Changes in Late EP Components to CS

A--habituation; B--1-20th combinations of CS and US; C--181-200 combinations; D--control; E--suppression (1-20 presentations of CS in 1 h after control); F--81-100 suppressions; G--281-300 suppressions. 1,2--marks of presentation of CS and US

phase III rose or dropped). The numerical data on the changes in EP amplitude are given in table 2.

In six experiments the response simultaneously was drawn in the focus of maximum activity of the auditory zone of the cortex and at the point 1.5-2 mm from it. Late EP components to the click in combination with CS and US underwent the same changes.

The averaged EP to the US (fig. 1. E) had besides the group of primary components a late negativeness and positiveness. The

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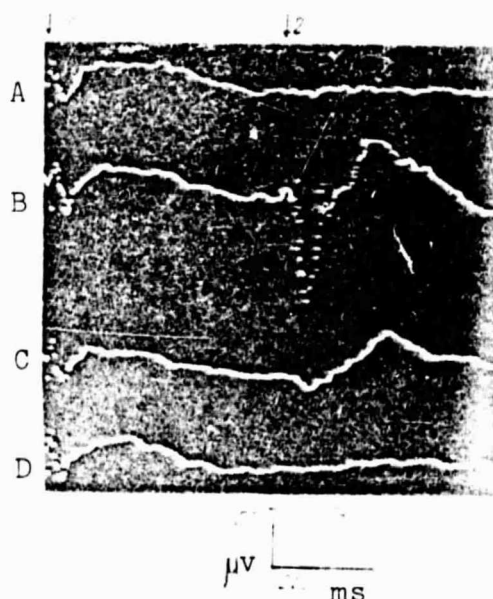


Figure 3. DEP in Somatosensory Zone of Cortex  
A--habituation; B--101-120 combinations of CS and US; C--Control;  
D--suppression (181-200). 1,2--marks of presentation of CS and US

latency of the peak of the late positiveness (phase II) after presentation of combinations increased on the average by 28%, the overall duration of the EP due to this rose on the average by 19%.

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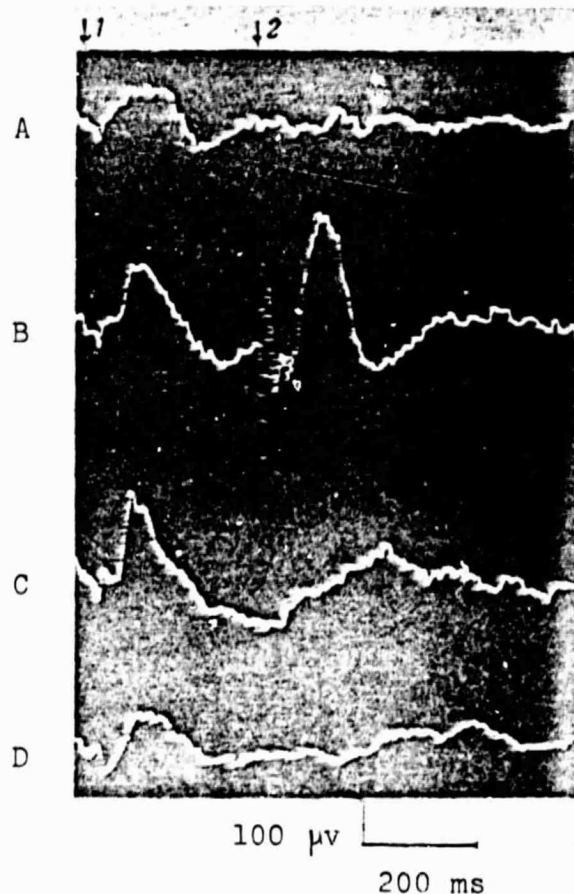
In the somatosensory and motor zones of the cortex the EP to the CS (fig. 1, B,C) in different experiments lasted from 100 to 350 ms depending on the duration of the late phases. The response to the CS in the somatosensory region after combinations of CS and US, as a rule, increased in amplitude (maximum by 200%). In the motor region it was not altered most often. If the changes were also recorded, they were expressed in a reduction of the early and increase in the late EP phases.

The EP to electrocutaneous stimulation are presented in fig. 1, F,G. The length of the response in both zones did not

significantly differ (250-400 ms). The amplitudes, latency of the peaks and overall length of the CS after presentation of the combinations naturally did not change. In a more precise analysis it was found that in the somatosensory region of the cortex with the selected strength of stimulation exceeding 6-10-fold the threshold, suppression of the response to the second in the series of stimuli observed in studying the cycles of restoration, often did not occur. In the reticular formation of the mesencephalon the complete duration of the EP to the CS (fig. 1, D) was 130-360 ms. After combination of CS and US phase II was not changed. Phase III was reduced, whereby in 27% of the experiments until complete disappearance. The FP to the electrocutaneous stimulation depending on the length of the late phases lasted 290-600 ms. The amplitude of the late phases after combination of CS and US most often was not altered, although individual cases of its increase and decrease were noted.

Delayed evoked potential. In the auditory zone of the cortex during the transmission of the US after 200 combinations of CS and US in 65% of the experiments a delayed evoked potential was recorded (DEP, fig. 2, D,E). With respect to time it emerged in the interval of the EP to the US. With respect to form the DEP in a number of experiments was similar to the late phases of the EP to the US. Depending on their extent the length of the DEP was 120-470 ms. The temporal shift in DEP in relation to the EP to the US in all points of contact where the DEP was recorded could reach 20-100 ms.

Most often 100-200 combinations of CS and US were required to generate the DEP. After it was generated it could be recorded even after the hour break in the experiment. In 71% of the experiments the DEP and changes in the late components of the EP to the CS were developed in parallel. Like the changes of the late phases of the EP to the CS the DEP was recorded not only in



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Figure 4. Suppression of DEP in Motor Zone of Cortex during Administration of Remiolan. A--habituation; B--161-180 combinations of CS and US; C--Control before preparation; D--Control within 5 min. after administration of 2 mg/kg of remiolan into vein; 1,2--mark of presentation of CS and US.

the focus of maximum activity, but also beyond its limits.

In the somatosensory zone of the cortex the DEP was recorded only in 14% of the experiments with amplitude 10-50  $\mu$ v (on the average 20% of the amplitude of phase II of the EP to the US). Figure 3, C presents one of the best DEP recorded in the somato- /860  
sensory region.

In the motor zone of the cortex the manifestation of DEP depended on the action of the myorelaxant--remiolan (fig. 4).

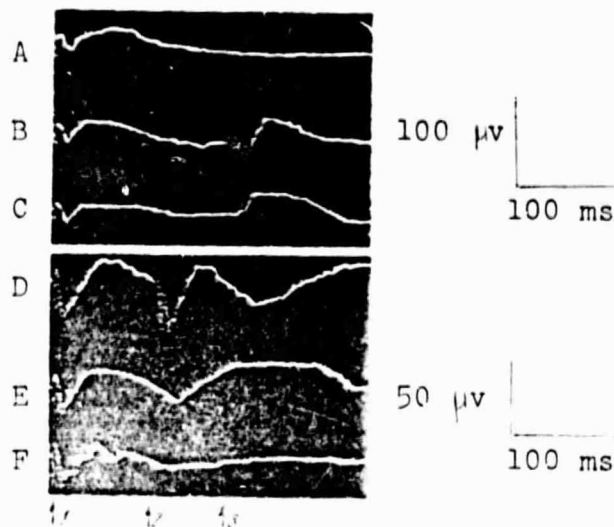


Figure 5. DEP in RF of Mesencephalon during "Relearning". A--habituation; B--181-200 combinations of CS and US with setting aside of 500 ms; C--Control; D--121-140 combinations of CS and US with setting aside of 300 ms; E--Control; F--Suppression (181-200 presentations of CS). 1--mark of CS; 2,3--marks of US

After the next administration of remiolan the DEP was reduced or completely blocked. With attenuation in the effect of the previous administration the DEP was recorded with amplitude 20-250  $\mu$ v with latency up to 50 ms. In these experiments, in addition to the DEP one could observe the conditioned reflex movement of the paw, however the DEP was recorded also without visible movement of the extremity.

In the reticular formation the DEP was observed in 44% of the experiments (fig. 5, C). Its amplitude was 20-100  $\mu$ v, latency in relation to the moment of US presentation was in different experiments 0-120 ms. As in other points of contact, the form of the DEP was often linked to the form of the late phases of response to the US.

It is important to note that only in 18% of the experiments with recording DEP in the auditory region was the DEP simultaneously

found in the RF. Here in both points of contact the DEP were weakly pronounced. Often the DEP was manifest in the RF in the first controls after combination of the CS and US, but after continuation of the combinations it disappeared. At this time the DEP emerged in the auditory region of the cortex. Another sequence of events was recorded where the DEP that emerged initially in the auditory cortex, then was replaced by a DEP in the RF of the mesencephalon.

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In four experiments after formation of the DEP with setting aside of the US from the CS of 300 ms a procedure was carried out of "relearning" for setting aside of 500 ms. All the experiments were successful, and after 200 combinations of CS and US a DEP emerged that was set aside by 500 ms. In two experiments in training with setting aside of 500 ms "relearning" was carried out for a setting aside of 300 ms. In one of them (fig. 5) the relearning was successful.

With the presentation of an isolated CS in the procedure of "suppression" the responses to the CS that were altered after the combinations were restored in 200-400 (sometimes up to 600) CS. The rate of DEP suppression depended on the length of "training." In "training" in 200 combinations the DEP usually disappeared after 100-200 isolated CS. A more rapid "suppression" of the DEP and EP to the CS occurred in a number of experiments during "relearning" for a great setting aside (10-30 combinations with a new time).

Twenty-nine percent of the experiments were unsuccessful in terms of DEP generation. Despite the satisfactory state of the animal that was controlled by EEG and pupils the DEP was not recorded in any of the contact points.

## Discussion of Results

The features of the employed model consist of the fact that the curarized animal cannot undertake actions directed towards avoiding the painful sensations. We are aware that such an experimental situation promotes the development of stress phenomena which interfere with the process of formation of a temporal link. They, apparently, are one of the causes of the relative difficulty in generating the DEP.

In principle the neurons of the cerebral cortex of the immobilized nonanaesthetized cat form conditioned reflex reactions to light and sound during reinforcement by electrical stimulation of the skin or sciatic nerve [3,11]. The main effect of the myorelaxant of Buchwald et al. [11] is considered to be the elimination of feedback from the gamma-afferents. In their experiments the animal trained under curare directly at the end of effect of the preparation did not execute a conditioned reflex motion. However, only several combinations of stimuli were sufficient to manifest the conditioned motion.

In our experiments also a conditioned reflex motion of the extremity was observed during transmission of the US, if the effect of remiolan passed. Here the DEP significantly rose in the motor cortex. The start of the muscular reaction, judging from the corresponding DEP of the motor zone of the cortex, was removed from the moment of CS presentation by a time linked to the engagement of the mechanism for counting time in the chain of spread of the signal from the region of the CS representation to the effector zone of the brain. This is also indicated by the correlation of the DEP in the auditory zone of the cortex and the RF to the time of the US. /862

That fact that in any collection of the 20 averagings we extremely rarely observed a DEP simultaneously in the auditory



cortical zone and the RF makes it possible only to note a fairly close negative link between these regions. Generally the neurophysiological experiments provide examples of both positive links with the RF [5], and negative [7]. It is not clear which role such a link plays in the organization of the DEP.

Thus, the question still remains open of whether the mechanism for counting time is linked to the specific structure of the brain, whether it reflects the circulation of the signal in the system of structures that participate in DEP generation, or can the delay of 300 and even 500 ms emerge parallel in several formations.

Some authors have described an increase in the EP to the CS during formation of the conditioned reflex [1,2,13,14,18], others--their decrease [6,15]. The opinion exists that the process of formation and reinforcement of the conditioned reflex is closely linked only to the change in the late phases of the EP to the CS [2]. Transformations of the amplitude of primary specific responses, probably, reflect only changes in the overall level of excitability of the brain [18,17].

In our experiments both an increase in the late EP components after combinations of CS and US, and their reduction were noted. The ambiguity of the results can be a consequence of the different initial state of the central nervous system of the animals. One should stress that fact that regardless of the direction of changes in EP in combination with the CS and US the isolated presentation of the CS in the procedure of "suppression" resulted in restoration of their initial amount.

The described electrophysiological correlates for the formation of the temporal link in the curarized animal, i.e., the DEP and changes in the late EP components have significant differences. The main difference, in our opinion, consists of the fact that



the late components of EP to the CS can only indicated the presence of the fact of temporary link. At the same time the DEP provides information about the delay in the unconditioned reaction, and in addition, in repeating in part of the experiments the form of the EP to the US it characterizes the reaction of the set of cerebral formations involved in the realization of the reflex. Here a comparison of the dynamics of the delayed responses makes it possible to judge the interrelationships of different levels of the brain during a conditioned reaction.

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